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BSRB, ABC Seminar rooms (Hybrid)

"Meet the neighbors: A universal technology for probing RNA-interactions and RNA-scaffolded subcellular compartments *in situ*"

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In the context of the living cell, very little RNA is naked. RNA molecules form complex, dynamic networks of molecular interactions that underlie a host of biochemical functions, and which are central to organizing subcellular compartmentalization. In humans, for example, RNAs are key determinants of chromatin folding, and they nucleate and scaffold a host of biomolecular condensates that collectively control cellular metabolic, epigenetic, and stress-signaling pathways. But, characterizing these structures—identifying the biomolecules within an RNA's subcellular microenvironment—remains technically cumbersome.

To address this challenge, I introduce oligonucleotide-mediated proximity-interactome mapping (O-MAP), a straightforward and flexible method for identifying the proteins, RNAs, and genomic loci near a target RNA, within its native cellular context. O-MAP uses programmable oligonucleotide probes to deliver proximity-biotinylating enzymes to a target RNA. These enzymes then pervasively label all nearby (~20 nm) molecules, enabling their enrichment by streptavidin pulldown. O-MAP induces exceptionally precise RNA-targeted biotinylation, and its modular design enables straightforward validation of probe pools and real-space optimization of the biotinylation radius, thus overcoming key technical challenges for the field. Moreover, O-MAP can be readily ported across different target RNAs and specimen types, including patient-derived organoids and tissue samples. And, O-MAP achieves this without complex cell-line engineering, using only off-the-shelf parts and standard manipulations.

Using a small cohort of model RNAs, we have developed a robust O-MAP toolkit for proteomic (O-MAP-MS), transcriptomic (O-MAP-Seq) and genome interaction (O-MAP-ChIP) discovery. O-MAP of the 47S-pre-rRNA—the long noncoding RNA that scaffolds the nucleolus—enabled a comprehensive "multi-omic" analysis of this subnuclear structure, and revealed hundreds of novel nucleolar protein-, RNA-, and chromatin interactions. O-MAP of XIST—the master regulator of X-chromosome inactivation—revealed novel RNAs that may play a role in this process, and unanticipated interactions between XIST and other chromatin-regulatory RNAs. Finally, targeting O-MAP to introns within a key cardiac developmental gene enabled unprecedented molecular dissection of a subnuclear compartment that would be impossible to purify biochemically.

Given these results, we believe that O-MAP will be a powerful tool for elucidating the mechanisms by which RNA molecules drive subcellular compartmentalization in time and space, with particular impact on our understanding of nuclear architecture. Moreover, with O-MAP's precision, flexibility, and ease, we anticipate its broad use in studying countless other RNA phenomena throughout biology, and as a clinical diagnostic- and discovery tool.